

PCR INS1

1- PCR mix

2,50 µl 10X buffer(Q BIOgene ; 1,5 mM MgCl₂)
 0,50 µl dNTP (20 mM total)
 0,20 µl primer INS1 UP (200 ng/µl)
 0,20 µl primer INS1 LP (200 ng/µl)
 0,20 µl primer NeoL (200 ng/µl)
 0,20 µl primer NeoR (200 ng/µl)
 0,20 µl Taq polymerase (Q BIOgene ; 5U/µl)
 19,00 µl distilled water

2- PCR reaction :

23,0 µl mix
 2,0 µl DNA (200ng/µl)

3- PCR program :

5 min	94°C	
30 sec	94°C	
30 sec	60 °C	35 cycles
1 min	72°C	
5 min	72°C	

4- Primers:

- Primer INS1 UP 5' CCAGCCAAGACTCCAGCGACTTTA 3'
 - Primer INS1 LP 5' CCCACACACCAGGTAGAGAGCCTCT 3'
 - Primer NeoL 5' GTGTTCCGGCTGTCAGCGCA 3'
 - Primer NeoR 5' GTCCTGATAGCGGTCCGCCA 3'

5- Amplification:

wild-type allele : 234 bp
 mutant allele: 555 bp